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# THE DISTRIBUTION OF SPORES OF *B. BOTULINUS* IN THE SOIL OF A RESTRICTED AREA IN CALIFORNIA. III \*

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Since the fatal outbreak of poisoning in 1919 in various parts of the United States due to the consumption of California olives which had been contaminated by *B. botulinus*, a widespread interest has been aroused, particularly in this state, as to the distribution of the spores of this bacillus. Aside from their desire to suppress this menace to the public health, various organizations, both national and local, financially interested in the growing and packing of fruits and vegetables, have greatly aided by grants of money the universities and public health laboratories in an intensive study of the different phases of this important problem. Burke<sup>1</sup> had found spores of *B. botulinus* on fruit and vegetables and the leaves of trees and plants in widely separated sections of California. It has been my privilege to read the manuscript of a monumental work on the distribution of the spores of *B. botulinus* in nature, by Meyer and Dubovsky, a portion of which appears in the form of several papers in the present issue of this journal. In one of these papers,<sup>2</sup> soils and other samples from many different localities in California, from the higher mountain regions, through the pasture lands of the foothills to the cultivated valleys, were studied, but in no case have many samples of soil been taken from several places within a comparatively restricted area. It is not within the scope of this paper to enter into a discussion of the many interesting features suggested by the work of these authors that such a study would present. The factors involved in the distribution of the two types of *B. botulinus* in soils, their origin, telluric differences and analogy to the distribution of *B. tetani* and other soil anaerobes are presented. The authors believe it possible that type A has its origin in the virgin soils of the mountain ranges, is swept down with such soils into the valleys, and that perhaps type B is merely a mutant of type A. The following report of the results

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\* This paper confirms and enhances the conclusions drawn in Paper II. It is a great pleasure to include it in the series of publications dealing with the distribution of *B. botulinus* in nature. K. F. Meyer.

<sup>1</sup> Jour. Bacteriol., 1919, 4, p. 5.

<sup>2</sup> The Distribution of the Spores of *B. botulinus* in California, this issue.

of some tests of soils for the presence of *B. botulinus*, which I undertook in the summer of 1921, may be of some interest in connection with their work.

In preparing for this study, some time was spent at the laboratory of the George Williams Hooper Foundation for Medical Research, University of California, where every facility was placed at my disposal for acquiring the technic employed by the workers in their investigations. In order that my results might be comparable to those

TABLE 1  
RESULTS OF AUTHOR'S EXPERIMENTS

1. S. walnut orchard, Carpinteria.....	10 miles east of Santa Barbara	<i>B. botulinus</i> , type A
2. F. lemon orchard, Summerland.....	8 miles east of Santa Barbara	<i>B. botulinus</i> , type A
3. G. olive orchard, Summerland.....	7 miles east of Santa Barbara	<i>B. botulinus</i> , type A
4. A. lemon orchard (twice), Montecito.....	6 miles east of Santa Barbara	<i>B. botulinus</i> , type A
5. C. vegetable garden, Montecito.....	5 miles east of Santa Barbara	<i>B. botulinus</i> , type A
6. Chicken droppings uncontaminated by soil (enclosure within above vegetable garden); 3 samples at different times	5 miles east of Santa Barbara	<i>B. botulinus</i> , weak toxin
7. Mixture fresh horse manure from 3 stalls, Bluebird Stables, Montecito	4 miles east of Santa Barbara	Negative
8. MacK. garden .....	City of Santa Barbara	<i>B. botulinus</i> , type A
9. Mixture wheat, cornmeal, rolled oats, sunflower seed, Union Commission Co.	City of Santa Barbara	Negative ( <i>B. welchii</i> infection)
10. Walnut orchard, Goleta.....	6 miles west of Santa Barbara	Negative
11. B. Ranch, bean field.....	8 miles west of Santa Barbara	<i>B. botulinus</i> , type A
12. Summit Santa Ynez Mountains.....	About 15 miles east Refugio Pass; elevation about 3,500 feet; sample taken (away from any trail) in thick underbrush	<i>B. botulinus</i> , type A

obtained in that laboratory, the technic thus acquired was adhered to and is fully described by Dubovsky and Meyer<sup>3</sup> in the first paper of their series.

The work described in this paper embraces a study of a few soils taken from a narrow strip of coast line about 18 miles long in Santa Barbara County, Calif., from Carpinteria on the east, through the city of Santa Barbara to the Bishop Ranch on the west. This strip averages about a mile and a quarter in width and is backed by the abruptly rising mountains of the Santa Ynez Range. The soils varied from light, sandy and rich loam to heavy clay.

<sup>3</sup> An Experimental Study of the Methods Available in the Enrichment, Demonstration and Isolation of *B. botulinus*, this issue.

Some preliminary studies were first undertaken with 3 strains of *B. botulinus* and a soil sample from the Yosemite Valley sent me by Dr. K. F. Meyer. I was able to confirm his findings for this soil (*B. botulinus*, type A isolated) as well as for a soil sample taken by him personally from an orchard near Santa Barbara. Each of my samples was made up of a mixture of soil from the surface to a depth of about 6 or 8 inches, often from a gopher mound, and was taken with the usual aseptic precautions and placed in containers sterilized at 170 for 2 hours.

My results are given in table 1.

It is to be noted that the toxin from the cultures of chicken droppings, though giving definite symptoms of *B. botulinus* poisoning in experimental animals, was too weak to type with antitoxin neutralization tests. Evidence has been given<sup>3</sup> that in such cases few spores exist in the material examined.

Cultures or tests were made of most of these soils several times, as severe tetanus symptoms often developed in the inoculated animals before the *B. botulinus* symptoms. In some cases, a protective dose of antitetanic serum was also given.

During the latter part of these experiments, liver was added to the beef heart peptic digest broth mediums, which gave a stronger toxin of *B. botulinus*, thus largely obviating the difficulty with the tetanus toxin.

It has been shown by this investigation that the cultivated soils of a narrow strip of coast line in Santa Barbara County, Calif., are heavily contaminated with the spores of *B. botulinus*, type A, as well as with those of *B. tetani* and that the virgin soil from the mountain range behind this strip of land also contains the spores of *B. botulinus*, type A.